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Liquid Phase Transition in the Postsynaptic Density?

Mary B. Kennedy[1,*] and **Tara L. Mastro**[1]

[1]Division of Biology and Biological Engineering, Mail Code 216-76, California Institute of Technology (Caltech), Pasadena, CA 91125, USA

An assembly of scaffold proteins termed the postsynaptic density (PSD) is attached to the postsynaptic membrane of excitatory glutamatergic synapses [1]. The scaffold serves to immobilize glutamate receptors in the membrane directly across from the position where glutamate is released from the presynaptic terminal. It also houses and organizes biochemical machinery whose job is to respond to particular patterns of electrical activity by increasing the strength of the synapse [2]. Synaptic strengthening helps to form new circuits that represent our experience [3]. These circuits are our memories. Biochemical machinery in the PSD strengthens the synapse by increasing the size of the PSD scaffold, the number of anchored receptors, and the size of the presynaptic active zone. A larger scaffold, more release sites, and more receptors means a stronger synapse, and vice versa.

A new study by Zeng *et al.* [4] explores the structural interaction between two abundant proteins in the PSD, and provides the first evidence that the structure of the PSD may involve liquid–liquid phase separation between PSD protein complexes and the cytosol. Such phase transitions have been proposed as driving forces in the formation of membrane-less cellular compartments such as the nucleolus [5,6].

The authors explored the interactions of fragments of synGAP, a multidomain enzyme in the PSD, and PSD-95, the principle scaffold protein in the PSD (Figure 1). SynGAP is a Ras and Rap GTPase-activating protein (GAP) that catalyzes the inactivation of Ras and Rap [7]. It is unusually abundant in isolated PSDs, nearly as abundant as PSD-95 itself, and inactivation of one copy of the synGAP gene in rodents or humans produces severe cognitive disability often with autistic features [8].

They first used isothermal calorimetry to examine interactions between the C-terminal 30 residues of synGAP (termed synGAP-PBM), which contain a PDZ ligand, and each of the individual PDZ domains of PSD-95, finding a weak interaction between the synGAP PDZ ligand and the first two isolated PDZ domains. By contrast, the interaction of the synGAP fragment with the third PDZ domain was slightly stronger (K_d = 27 μM), and was made even stronger (K_d = 1.8 μM) by including an additional 11 residues that form an α-helix beyond the C-terminus of the PDZ domain. A crystal structure revealed that the extended α-

*Correspondence: kennedym@its.caltech.edu (M.B. Kennedy).

helix after the PDZ3 domain interacts with residues immediately upstream of the canonical five-residue PDZ ligand in synGAP-PBM.

Zeng *et al.* then examined a larger stable fragment of synGAP comprising the 90 residue coiled-coil domain upstream of the C-terminal PDZ ligand (synGAP-CC-PBM; Figure 1). Biophysical measurements of the size of the synGAP-CC-PBM fragment in solution indicated that it forms a stable trimer. A crystal structure of the coiled-coil domain revealed a parallel coiled-coil trimer that likely mediates the trimerization of the entire fragment.

The most interesting finding arose when the authors examined the interaction of the synGAP-CC-PBM trimer with an expanded fragment of PSD-95 comprising the third PDZ domain, the SH3 domain, and the entire guanylate kinase-like domain (termed PSD-95-PSG; Figure 1). The two were found to interact with high affinity (K_d = 140 nM), measured by isothermal calorimetry, and with a fixed stable 3:2 stoichiometry: one synGAP-CC-PBM trimer binds to two PSD-95-PSG fragments. Furthermore, when the purified fragments were mixed at room temperature at concentrations above 100 μM, the authors noticed that the solution turned opalescent. Examination under the light microscope revealed spherical liquid droplets ranging from 20 to 50 μm in diameter suspended in the aqueous solution. The droplets form a distinct phase that can be sedimented from the aqueous phase by centrifugation. Mutations of synGAP-CC-PBM that remove or disrupt the PDZ ligand and mutations within the coiled-coil domain that block the formation of the trimer both abolished formation of the stable 3:2 complex and also abolished the liquid phase separation. Thus, an intact PDZ ligand in synGAP, and association of the synGAP fragment into a trimer, are both required for formation of a structure that separates into a distinct liquid phase.

This behavior is reminiscent of the liquid phase transitions that are believed to drive formation of ‘membrane-less’ intracellular compartments such as the nucleolus and the centrosome. These compartments seem to function as specialized, highly-dynamic ‘reaction vessels’ in which a set of reactants are concentrated and loosely organized to drive the assembly of protein machines. Ribosomes are assembled in the nucleolus [9]; spindles form and are anchored at the centrosomes [10].

The phase separation observed by Zeng *et al.* involves only a small fragment of synGAP interacting with the PSG fragment of PSD-95. Others have reported additional high-affinity interactions of full-length synGAP with the first two PDZ domains of PSD-95, which are not present in PSD-95-PSG. Thus, regions of synGAP in addition to those contained in the synGAP-CC-PBM fragment contribute to complex formation with PSD-95 [11]. Furthermore, phosphorylation of several sites within the disordered domain of synGAP has been shown to modulate the affinity of the synGAP PDZ ligand for each of the PDZ domains [11]. Thus there is still much to be learned about the structure and regulation of complexes between the two full-length proteins *in vivo* and their potential for liquid phase separation.

Zeng *et al.* did show that regulated movement of overexpressed full-length fluorescent synGAP into and out of the synapse in cultured neurons is reduced by mutations that disrupt

the 3:2 complex *in vitro*. However, because the nature of any complex formed between the two full-length proteins has not yet been established, the structural underpinnings of the reduction in synaptic localization *in vivo* are not yet defined. Nonetheless, the striking physical chemical behavior of the complex between synGAP-CC-PBM and PSD-95-PSG hints at structural principles involving liquid phase separation that have not been previously considered for the PSD scaffold.

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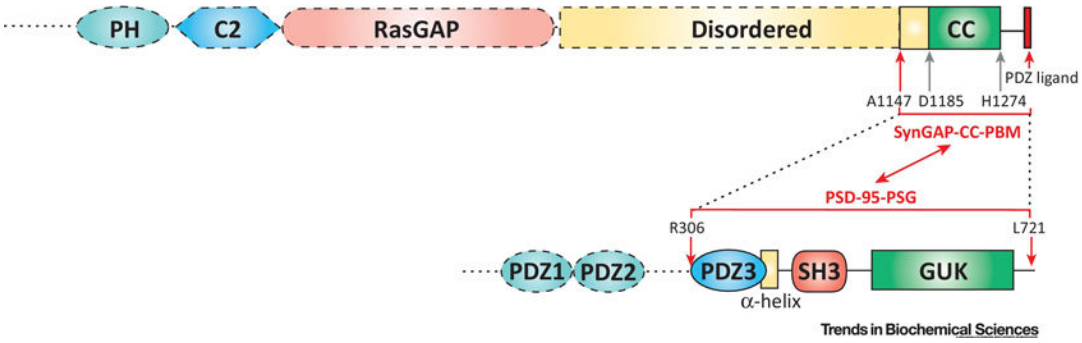

Figure 1.

Scaled Domain Diagrams of SynGAP and PSD-95. The domains surrounded by solid lines are contained in the fragments synGAP-CC-PBM and PSD-95-PSG that form the ordered 3:2 complex as described in the text. The domains surrounded by dashed lines are not included in the fragments. The C2 and RasGAP domains of synGAP mediate its GTPase-activating activity toward Rap (C2 plus RasGAP) and Ras (RasGAP alone) [12]. The disordered domain of synGAP contains several phosphorylation sites that regulate the specificity of the GAP domain and the affinity of synGAP for the PDZ domains of PSD-95 [7,11,13]. Abbreviations: CC, coiled-coil domain; GUK, guanylate kinase homology domain; PBM, PDZ domain binding motif; PDZ domain, named from PSD-95, Discs large, and ZO-1; PH, pleckstrin homology domain; PSD-95, postsynaptic density 95 kDa; PSG, PDS/SHG3/GUK domains; RasGAP, Ras-like GTPase-activating protein domain; SH3, Src homology 3 domain; SynGAP, synaptic Ras GTPase activating protein 1.